High ambient glucose is effect neutral on cell death and proliferation in human proximal tubular epithelial cells

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High ambient glucose is effect neutral on cell death and proliferation in human proximal tubular epithelial cells. Am J Physiol Renal Physiol 289: F401–F409, 2005. First published April 12, 2005; doi:10.1152/ajprenal.00408.2004.—In vitro models of diabetic nephropathy that assess the role of hyperglycemia on proximal tubular cell turnover commonly compare cells in a high-glucose medium (25 or 30 mM) with a low-glucose medium (5 to 6.1 mM). Any cellular growth changes observed are usually attributed to the effect of high glucose. We hypothesize that in such experiments, glucose concentrations in the low-glucose medium may decline during the course of the experiments to levels that inhibit cell growth leading to the comparative conclusion that high glucose induces hyperplasia and/or hypertrophy. In this study, primary cultures of human proximal tubular epithelial cells (PTEC) and immortalized HK-2 cells were exposed to low (5 mM) or high (17, 30, or 47 mM) glucose for up to 6 days (PTEC) and 48 h (HK-2). When culture media were not replenished, low glucose induced a significant increase in necrosis and release of lactate dehydrogenase and a decrease in proliferation, metabolic activity, and protein content without any changes in apoptosis. High-glucose media failed to induce any of these changes. Glucose was undetectable in the low-glucose culture medium after 72 h. No significant differences were observed between any of the treatment groups when culture media were replenished daily. We conclude that regular replenishment of culture media is necessary to prevent the emergence of artificial and misleading differences between high- and low-glucose groups. The current knowledge of the pathophysiology of high glucose based on cell culture systems may need to be reevaluated.

apoptosis; diabetic nephropathy; hyperglycemia; necrosis; proximal tubule

DIABETES IS THE LEADING CAUSE of end-stage renal disease in the Western world (18). Apart from genetic susceptibility and obesity, chronic exposure of renal tissue to elevated blood glucose levels has been suggested to be an essential factor in the development of overt diabetic nephropathy (DN) (25, 26). DN is primarily a disease of the glomerulus. The thickening of the glomerular basement membrane and the expansion of extracellular matrix significantly reduce the surface area available for filtration and gradually occlude the capillary lumen. This leads to hyperfiltration of residual filtration surfaces and eventually microalbuminuria (13). However, nephromegaly in the early stages of diabetes and the correlation of tubulointerstitial pathology rather than glomerular pathology with declining renal function in DN (2, 3, 24, 29) suggest the involvement of the tubulointerstitium. Although the etiology of the tubulointerstitial pathology in DN is not fully understood, much attention has focused on the role of high glucose per se. It is now accepted that tubular cells are a primary target of hyperglycemia and that chronic exposure to elevated blood glucose levels contributes to the tubulointerstitial changes seen in overt DN (3, 25, 26).

Much of the information on a direct role of high glucose on the tubulointerstitium is derived from in vitro studies. Proximal tubular epithelial cells (PTEC) exposed to high-glucose concentrations (25 to 30 mM) are reported to undergo hypertrophy (16, 30) or hyperplasia (10, 11). It has been suggested that such increases in cell growth correspond with the tubulointerstitial hyperplasia and hypertrophy observed in animal models shortly after the onset of diabetes (10, 11, 22). In contrast, other studies (1, 15, 27, 28) show that exposure of PTEC to high glucose (25 to 30 mM) induces cell death by apoptosis, offering a possible explanation of the mechanism for tubular atrophy observed in advanced stages of DN.

In vitro models of DN using PTEC traditionally compare cells in a low-glucose medium (5 to 6.1 mM) with cells in a high-glucose medium (25 or 30 mM). Any observed changes in growth characteristics are then attributed to the effect of the high-glucose medium on the cells under investigation. However, it is well known that most cells require glucose-enriched media to survive in vitro (10 to 25 mM; 17 mM for PTEC). Thus experiments that consider a glucose concentration of 5 to 6.1 mM to be normoglycemic (the normal range for glucose is 3.0 to 7.8 mM) may in fact be “low” for these cells and a glucose concentration considered to be high (25 to 30 mM) may be not much higher than their “optimum” glucose concentration in vitro. Therefore, experimental models that use a medium with low-glucose concentrations as controls and then compare the growth effects of PTEC with a medium containing high-glucose concentrations may be fundamentally flawed.

We hypothesize that glucose concentrations in the low-glucose medium (5 to 6.1 mM) may decline with the passage of time and reach levels that do not support growth and possibly induce cell death. High-glucose media on the other hand (25 to 30 mM) may still have glucose levels capable of sustaining growth despite substantial reductions in glucose concentrations with time. Regular replenishment of culture medium may overcome the potential problem of glucose depletion with the result that differences in cell growth or death between cells exposed to low- and high-glucose concentrations...
are not observed. Based on these hypotheses, we reevaluated the role of high glucose on cell death (apoptosis and necrosis) and cell growth (proliferation) of primary cultures of human PTEC and HK-2 cells, an immortalized human PTEC line.

**MATERIALS AND METHODS**

The use of human PTEC in this study was approved by the Human Research Ethics Committee of the Royal Brisbane and Women's Hospital, Brisbane, Australia. Written informed consent was obtained from patients before nephrectomy for the collection of cortical tissue samples.

**Chemicals.** Chemicals for cell culture were obtained either from Sigma (Castle Hill, NSW, Australia), GIBCO (Invitrogen, Carlsbad, CA), or Roche (Roche Diagnostics) unless stated otherwise.

**Isolation and culture of human PTEC.** PTEC were isolated and cultured following the method of Glynn (4). Renal cortical tissues from the normal region of nephrectomy samples were collected in precooled (4°C) Hank's balanced salt solution (HBSS) containing penicillin (50 U/ml), streptomycin (50 μg/ml), and amphotericin B (0.125 μg/ml). After the capsule was removed, the extreme cortex was cut into small pieces and centrifuged at 200 g for 5 min at 4°C. The supernatant was discarded and the tissue fragments were suspended in 1 mg/ml (in HBSS) of type II collagenase (Worthington Biochemical) and incubated for 1 h at 37°C. The digest was passed through a 100-μm sieve followed by a 40-μm sieve (Beckton Dickinson). The sieved cells were centrifuged (200 g, 5 min, 4°C) and seeded into 75-cm² tissue-culture flasks that had been coated with collagen S and FBS. The cells were grown in serum-free medium which was DMEM and Ham’s F12 (DMEM/F-12; glucose concentration, 17 mM) containing 15 mM HEPES buffer, 1-glutamine, and pyridoxine hydrochloride. The medium was supplemented with epidermal growth factor (10 ng/ml), insulin (10 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), hydrocortisone (36 ng/ml), triiodothyronine (4 μg/ml), penicillin (50 U/ml), streptomycin (50 μg/ml), and amphotericin B (0.125 μg/ml). The cells were incubated at 37°C in humidified 5% CO₂ in air. Experiments were conducted on passages 2 and 3 cells in medium that was devoid of growth supplements.

**Morphological, biochemical, and immunohistochemical tests.** The PTEC have polarized morphology with numerous apical microvilli and tight junctions. The cells expressed the brush-border enzymes alkaline phosphatase activity and γ-glutamyl transferase, were immunopositive for cytokeratin, displayed glucose-dependent glucose uptake, and exhibited CAMP stimulation by parathyroid hormone but not by arginine vasopressin.

**Culture of HK-2 cells.** HK-2 cells were cultured in DMEM/F-12 (17 mM glucose) supplemented with 10% FBS, penicillin (50 U/ml), streptomycin (50 μg/ml), and amphotericin B (0.125 μg/ml). Experiments were conducted in FBS-free medium.

**Preparation of glucose media.** Unlike in vivo, both PTEC and HK-2 cells require and are routinely cultured in glucose-enriched media (usually 17 mM). Because this is actually “hyperglycemia” in vivo, the choice of ambient glucose concentration in the culture medium that can be considered the “control” becomes critically important. To address this issue, two sets of experiments were designed. In the first set, 17 mM glucose (the medium in which PTEC were isolated, cultured, and characterized) was designated the control and, in the second set, a medium constituted with 5 mM glucose (as cited in most of the literature) served as the control.

To prepare culture media containing a range of glucose concentrations, several combinations of culture media were considered. If required, glucose concentrations were augmented by the addition of D-glucose (Sigma); medium A: DMEM/F-12 containing 17 mM glucose (GIBCO cat. no. 11330–032; the medium used for the isolation, culture, and characterization of PTEC); medium B: DMEM/F-12 with 47 mM glucose, prepared by adding glucose to medium A; medium C: DMEM without glucose (GIBCO cat. no. 1196–025); medium D: F-12 with 10 mM glucose (GIBCO cat. no. 11765–054); medium E: DMEM/F-12 with 5 mM glucose, prepared by mixing equal volumes of medium C and medium D; medium F: DMEM/F-12 with 30 mM glucose (medium E with additional glucose); medium G: DMEM with 5 mM glucose (medium C with additional glucose); and medium H: DMEM with 30 mM glucose (medium C with additional glucose).

Because the media combinations G and H do not contain F12, the effect of the presence (or absence) of F-12 on cell growth and death parameters was investigated in our pilot studies. A comparison was made between cells grown in DMEM/F-12 with 5 or 30 mM glucose (medium C and medium F, respectively) and cells grown in DMEM at the same glucose concentrations but without F-12 (medium G and H, respectively). As the results showed no significant differences between the two groups, the second set of experiments that considered 5 mM glucose as the control was carried out using the latter (DMEM without F-12).

**Experimental protocol for primary cultures of PTEC.** PTEC (5 × 10⁴ cells/ml) were cultured in serum-free DMEM/F-12 supplemented with growth factors in 24-well culture plates. Only cells from passages 2 and 3 were used for experiments. Subconfluent cells were washed and incubated in growth supplement-free medium (medium A) for 24 h. After 24 h, the cells were incubated in medium A (17 mM control) and medium B (47 mM high glucose) and comparisons were made between these two groups. In a parallel second set of experiments, PTEC were exposed to medium G (5 mM control) and medium H (30 mM high glucose) and a comparison was made between these two groups. The experiments were carried out for 24 h, 48 h, 72 h, and 6 days. The volume of culture medium in each well was 1 ml. Mannitol was used as osmotic control. Positive controls were chosen on the basis of their known efficacy with respect to the parameter under investigation.

**Experimental protocol for HK-2 cells.** HK-2 cells (1 × 10⁵ cells/ml) were cultured in 24-well plates in DMEM/F-12 (17 mM glucose) supplemented with 10% FBS. Subconfluent cells were exposed to FBS-free culture media containing various concentrations of glucose and comparisons made as described above. The experiments were carried out for 24 and 48 h.

**Apoptosis and necrosis measurement by flow cytometry.** Apoptosis and necrosis were determined using a commercially available kit (Annexin-V-FLUOS Staining Kit, Roche Diagnostics). In brief, after the appropriate treatment periods, nonadherent cells were pelleted and added to trypsinized and pelleted adherent cells. The cells were resuspended in 100 μl of binding buffer containing fluorescein isothiocyanate (FITC)-conjugated annexin-V and propidium iodide (PI) and incubated at room temperature for 15 min. After the incubation period, 300 μl of binding buffer were added and the cells were analyzed in a FACS Calibur (Beckton Dickinson) using 488-nm excitation and a 515-nm band pass filter for fluorescein detection and a filter >600 nm for PI detection. Ten thousand events were recorded from each treatment group.

**Apoptosis measurement using in situ end labeling.** Apoptosis was also determined using a commercially available ApoTag Peroxidase kit (Serologicals) that is based on the TUNEL (TdT-mediated nick end labeling) method. Briefly, PTEC were grown on Thermofax coverslips (Nalge Nunc International) in 24-well plates. After the appropriate treatment periods, cells were washed in PBS and fixed in 1% paraformaldehyde for 10 min at room temperature. The fixative was washed away and the cells were postfixed in precooled ethanol: acetic acid mixture (2:1 vol/vol) for 3 min at −20°C. After the endogenous peroxidase with 3.0% hydrogen peroxide in TBS was
BrdU labeling solution was removed and 200 μl of BrdU labeling solution were added to each well and incubated at 37°C. The selected treatment periods, 10 μl of a commercially available kit (Cell Proliferation ELISA, BrdU Colorimetric kit, Roche Diagnostics) were added to each well and incubated for 15 min at room temperature. After the BrdU labeling solution was removed and 200 μl of FixDenat solution were added to each well and incubated for 15 min at room temperature. The secondary antibody conjugate was removed and the cells were rinsed three times with the washing solution. Substrate solution was added (100 μl), incubated at room temperature for 20 min, and the color was measured in a microplate reader at 370 nm with a reference wavelength of 492 nm.

Cell proliferation assay using 5-bromo-2′-deoxyuridine. Cell proliferation was determined using a commercially available kit (Cell Proliferation ELISA, BrdU Colorimetric kit, Roche Diagnostics). Cells grown in 96-well culture plates were incubated in 100 μl of respective experimental glucose media. Ninety minutes before the selected treatment periods, 10 μl of 5-bromo-2′-deoxyuridine (BrdU) labeling solution were added to each well and incubated at 37°C. The BrdU labeling solution was removed and 200 μl of FixDenat solution were added to each well and incubated for 15 min at room temperature. After the FixDenat solution was removed, 100 μl of anti-BrdU-POD working solution were added to each well and incubated for 60 min at room temperature. The secondary antibody conjugate was removed and the cells were rinsed three times with the washing solution. Substrate solution was added (100 μl), incubated at room temperature for 20 min, and the color was measured in a microplate reader at 370 nm with a reference wavelength of 492 nm.

Metabolic assay using MTT. Metabolic activity was measured using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). In brief, cells were grown in 96-well culture plates in 100 μl of the respective experimental glucose media. Ninety minutes before the selected treatment periods, 10 μl of MTT solution (5 mg/ml in PBS) were added to each culture and incubated at 37°C. The culture medium was removed and the purple crystals formed were dissolved in 150 μl of 0.1 N HCl in isopropanol. The absorbance was measured at 570 nm with a background correction of 690 nm.

Protein assay. Cells were grown in 96-well plates for selected time periods. The culture medium was removed and cells were washed twice with PBS. The cells were lysed in 50 μl of RIPA buffer (0.5% sodium deoxycholate, 0.1% SDS, and 1% nonidet P-40 in PBS) at 4°C. BCA protein assay reagent mix (Pierce) was added (150 μl) and the color was developed for 20 min at room temperature and read in a microplate reader at 562 nm. Bovine serum albumin dissolved in RIPA buffer was used as standard.

Glucose concentrations in the culture medium. PTEC and HK-2 cells were exposed to DMEM containing 5, 17, 30, and 47 mM glucose. The culture media were prepared by adding appropriate concentrations of glucose to DMEM without glucose (medium C). The glucose concentration in the medium was measured every 24 h until the end of the study using the Roche/Hitachi Modular system (Roche, Mannheim, Germany). To find out whether glucose undergoes degradation during the course of the experiment, these culture media were also incubated in 24-well plates without cells. The glucose concentrations were measured every 24 h as above.

Glucose replenishment experiments. PTEC and HK-2 cells were exposed to 5, 17, 30, and 47 mM glucose in 96-well plates. In one set of experiments, the culture media in all four treatment groups were replenished every 24 h and in the other set, the culture media were not replenished until the end of the study. Cell proliferation, metabolic activity, and protein content in PTEC and HK-2 cells were measured at 6 days and 48 h, respectively. Lactate dehydrogenase (LDH) release (a measure of necrosis) was measured using the Roche/Hitachi Modular system (Roche).

Statistical analysis. Experiments involving PTEC were carried out three times in duplicate, using cells obtained from three different nephrectomy samples. Experiments with HK-2 cells were carried out six times. The results were expressed as means ± SE. Comparisons among groups were performed using one-way ANOVA with Tukey’s post hoc analysis. Analyses were performed using Graphpad InStat software (San Diego, CA). P < 0.05 was considered significant. Significant changes in the parameters, if present, were observed only on day 6 for PTEC and at 48 h for HK-2 cells. Therefore, the results obtained at 48 and 72 h for PTEC are not included.

RESULTS

Apoptosis. Exposure of PTEC or HK-2 cells to various concentrations of glucose did not result in any significant changes in apoptosis. ##P < 0.01 and ###P < 0.001 vs. other groups.

**Fig. 1.** Effect of glucose on apoptosis of primary cultures of human proximal tubular epithelial cells (PTEC, A) and HK-2 cells (B) determined by flow cytometry. The 2 groups at each time point represent 2 different experimental conditions. Left: 4 columns show results obtained using medium A (DMEM/F12 with 17 mM glucose) and medium B (47 mM glucose). Right: 3 columns represent results obtained using medium C (DMEM with 5 mM glucose and medium H (30 mM glucose). Appropriate concentrations of mannitol were used as osmotic control. A plasma protein fraction (30 – 440 kDa, 10 mg/ml) which induces apoptosis in primary cultures of PTEC (14) was used as a positive control. Hydrogen peroxide (H2O2; 1 mM) was used as positive control for HK-2 cells. Treatment of PTEC or HK-2 cells with high glucose did not induce apoptosis, whereas the positive controls induced significant increases in apoptosis. ##P < 0.01 and ###P < 0.001 vs. other groups.
differences in apoptosis as measured by annexin binding (Fig. 1, A and B). The TUNEL method in combination with morphological verification also failed to show any significant changes in apoptosis in response to high glucose compared with low glucose (Fig. 2, A and B).

**Necrosis.** No significant changes in necrosis in response to high experimental glucose concentrations (47 compared with 17 mM) were observed in PTEC or in HK-2 cells during the study (Fig. 3, A and B). However, on day 6, PTEC exposed to low glucose (5 mM) showed a significant increase in necrosis compared with the groups treated with high glucose (30 mM; Fig. 3A).

**Cell proliferation and metabolic activity.** High ambient glucose failed to induce any significant differences in cell proliferation (Fig. 4, A and B) and metabolic activity (Fig. 5, A and B) in both PTEC and HK-2 cells throughout the study period. However, on day 6 in PTEC and at 48 h in HK-2 cells, low glucose (5 mM) induced a significant reduction in cell proliferation (Fig. 4, A and B) and metabolic activity (Fig. 5, A and B) compared with the 30 mM glucose group.

**Protein content.** PTEC exposed to 5 mM glucose showed a significant reduction in protein content on day 6 without any changes at other time points (Fig. 6A) compared with 30 mM groups (Fig. 6A). Exposure of HK-2 cells to various concentrations of glucose failed to induce any significant differences in protein content (Fig. 6B).

**Glucose concentrations in the medium.** Measurement of glucose concentrations of the culture medium in which the cells were grown showed a linear decline with time in the four
groups (Fig. 7, A and B). By 48 h, the glucose concentration in the culture medium containing 5 mM glucose had fallen to 1.2 ± 0.4 mM for PTEC (1.23 ± 0.03 for HK-2 cells) and by 72 h the glucose concentration was less than 0.3 mM. Such a decrease in glucose concentration was not observed in the culture media that were incubated without cells (Fig. 7 C).

**Replenishment of glucose.** Measurement of cell proliferation, metabolic activity, protein content, and LDH release was carried out on day 6 for PTEC and at 48 h for HK-2 cells. These time points were chosen because significant changes in these parameters were previously observed at these time points (Figs. 4 to 6). When the culture media were replenished every day, no significant differences in cell proliferation (Fig. 8), metabolic activity (Fig. 9), and protein content (Fig. 10) between the low (5 mM)- and high-glucose groups (17, 30, and 47 mM) were observed. When the media were not replenished, cells in the 5-mM group showed significant reproducible changes in these growth parameters (Figs. 8–10), similar to those observed previously (Figs. 4–6). Thus, when the medium was not replenished, PTEC showed a decrease in cell proliferation, metabolic activity, and protein content when compared with respective glucose groups in which the medium was replenished. The role of low glucose (5 mM) in inducing significant necrosis (as measured by propidium iodide uptake; Fig. 3A) was confirmed by the observation of increased LDH release when PTEC were cultured in nonreplenished media containing 5 mM of glucose (Fig. 11).

**DISCUSSION**

The selection of the control group is very important when studying the role of hyperglycemia in the behavior of PTEC in...
Control conditions in many published studies have been defined as between 5 and 6.1 mM glucose to reflect the in vivo concentrations of human plasma (3 to 7.8 mM). However, these normoglycemic concentrations of glucose are not optimal for human PTEC in vitro, which are routinely isolated and cultured in media with higher glucose concentrations (4, 10, 11). For example, DMEM/F12 medium is usually constituted with 17 mM glucose. An ideal model would require these cells to be isolated and grown under normoglycemic conditions (5 mM glucose or within the normal range of 3.0 to 7.8 mM). But this is not done routinely nor has it been a feature of earlier publications. It is doubtful whether these cells can be isolated from nephrectomy samples, cultured, and characterized in such low-glucose concentrations, and our attempts to isolate human PTEC in 5 mM glucose from nephrectomy samples were unsuccessful. Furthermore, HK-2 cells are successfully grown in 17 mM glucose (DMEM/F-12 or DMEM alone supplemented with 10% FBS). In our laboratory, these cells show excessive vacuolations and altered morphology (which are markers of cell stress) when grown in 5 mM glucose. This substantiates what is well known, namely, that primary cultures of human PTEC and HK-2 cells (and most cell types) require higher “diabetic” concentrations of glucose for “normal” cell survival and growth in vitro. The question therefore arises as to what controls should be used in in vitro models of DN using PTEC and HK-2 cells. To address this question, we considered 5 and 17 mM glucose concentrations as controls and compared
We found that high ambient glucose concentrations were effect neutral and induced neither apoptosis nor necrosis of PTEC or HK-2 cells. This contrasts with previous in vivo (8) and in vitro (1, 15, 27, 28) studies that attributed a direct role for high glucose in inducing apoptosis of PTEC. For example, Ishii et al. (8) found that intravenous loading of rats with extremely high concentration of glucose (550 mM or 10% glucose solution) for 5 h produced a significant increase in DNA fragmentation and the ladder-like pattern characteristic of apoptosis in rat proximal tubular cells. With respect to in vitro studies, exposure of mouse PTEC to 25 mM glucose induced a significant increase in apoptosis as early as 24 h (15). This was associated with an increased expression of the proapoptotic Bax gene and a decreased expression of the anti-apoptotic Bcl-2 gene (15). LLC-PK1 cells (porcine proximal tubular epithelial cell line) exposed to 25 mM glucose (1) and HK-2 cells treated with 30 mM glucose (27, 28) have been reported to undergo apoptotic cell death at 48 h. We were unable to find any significant changes in apoptosis of primary cultures of human PTEC and HK-2 cells in response to a range of glucose concentrations.

To our knowledge, there are no previous reports on the role of high glucose on apoptosis of human PTEC in primary culture. We have no explanation as to why we did not observe apoptosis in HK-2 cells in response to high glucose as reported by others (27, 28), despite the use of similar methods. Methodological error can be ruled out because the positive controls in our studies produced significant apoptosis in both cell types. However, there are emerging reports that suggest that high glucose enhances survival rate in pancreatic β-cells as well as neurons (20, 23), that high glucose inhibits apoptosis and necrosis of neural, gastric, and cardiac cells (5–7, 19, 21), and that high glucose protects cardiac cells against hypoxic insults (12, 17). Interestingly, high ambient glucose did not induce necrosis in any of the treatment groups in the present study, but we did observe a significant increase in necrosis of human PTEC exposed to low glucose on day 6.

The role of high glucose in hyperplasia and hypertrophy of PTEC is also controversial. Mouse (30) and rabbit (16) PTEC showed a significant reduction in cell proliferation and increase in protein content, suggesting that hypertrophy is the predom-
Inhibitory response to high ambient glucose. Studies of human PTEC exposed to 25 mM glucose for 6 days have reported that the cells undergo significant proliferation without much change in protein content (10, 11). These studies concluded that hyperplasia, not hypertrophy, is the major response to high glucose (10, 11). Although we observed no changes in cell growth characteristics in the high-glucose groups, human PTEC exposed to 5 mM glucose for 6 days showed a decrease in cell proliferation, metabolic activity, and protein content. There was a similar decrease in proliferation and metabolic activity of HK-2 cells at 48 h but the protein content was unchanged.

To determine whether the observed changes in cell proliferation, metabolic activity, and protein content were due to an increase in response to high glucose as reported in the literature (10, 11) or a decrease in response to low glucose, further experiments were carried out using human PTEC and HK-2 cells. Analysis of glucose concentrations in the media in which the cells were grown showed a steady decrease in glucose concentration during the course of the experiments, reaching undetectable levels in the 5-mM glucose group (Fig. 7). This decrease was not due to the degradation of glucose in the culture medium, but solely due to utilization by the cells. These findings support our hypothesis that the reduced cell growth and increased cell death observed in the low-glucose medium (5 mM) were the consequence of glucose depletion such that cell survival was compromised rather than high glucose inducing hyperplasia and/or hypertrophy.

To verify this conclusion, we examined the effects of replenishing the culture media constituted with the experimental glucose concentrations every day. Interestingly, when the culture media were replenished, no significant differences in any of the indexes of cell death (LDH release) or growth (proliferation, metabolic activity, and protein content) were observed between high- and low-glucose groups. Less cell proliferation, lower metabolic activity, and protein content were again observed in the nonreplenished low-glucose group (5 mM) compared with the nonreplenished high-glucose groups. The significant increase in LDH release together with necrosis in the nonreplenished low-glucose group clearly shows that low glucose rather than high glucose is responsible for the observed changes.

In summary, this study does not support the notion that high glucose induces either cell growth or cell death in primary
cultures of human PTEC or HK-2 cells, at least under the experimental conditions we employed. Irrespective of the glucose concentration in the medium in which these cells are routinely grown, it is imperative to interpret the results of in vitro models of DN in the context of changes in glucose concentrations during the course of the experiment. Ignoring the longitudinal changes in glucose concentration during an experiment could lead to the misinterpretation that high glucose induces growth of PTEC. The current knowledge of the pathophysiology of high glucose based on cell culture systems may need to be reevaluated.

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GRANTS

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